Chemopotentiation by Low Dose-Fractionated Radiation Therapy
Chemopotentiation by LDFRT is a novel treatment paradigm that allows for the safe use of full-dose systemic chemotherapy in combination with low-dose fractionated radiotherapy.

This approach can provide survival advantages in malignancies that have disseminated into tissues surrounding the primary tumor.

- Where the low-dose radiation sensitizes the tumor to subsequent chemotherapy, resulting in increased primary site and nodal site response rate

However, the clinical benefit of this combined modalities have been variable probably due to the fact that the molecular mechanisms underlying chemopotentiation by LDFRT are still ill defined.
Disseminated intra-abdominal disease is present in 10–30% of GI cancer cases and is a frequent finding in patients who develop recurrent cancer.

Natural history studies have established a 6-month median survival in this group of patients.

Although GI carcinomas are known to be radiosensitive tumors, it has been a challenge to use full doses of chemotherapy in combination with standard doses of radiation therapy due to the increased toxicity.

Whole abdominal radiotherapy (WART) has been used in cases of GI cancer with disseminated intra-abdominal disease.

However, the main shortcoming of WART is the inability to combine it with full-dose chemotherapy, which is a significant drawback in the attempt to eradicate disseminated micrometastatic disease.
1: Can Chemopotentiation by LDFRT be applied to disseminated intra-abdominal Gastric cancer?

2: If so, what are the molecular mechanisms underlying this effect?
LDFRT can induce Hyper-radiosensitivity

Traditionally, radiation doses greater than 120 cGy have been used in radiotherapy because lower doses were thought to be ineffective for tumor therapy.

LQ: $\alpha/\beta$

However, we now know that LDFRT can produce hyper-radiosensitivity (HRS), a phenomenon where cells undergo apoptosis at radiation doses as low as 15 cGy, in a number of proliferating cells.

- This approach can provide survival advantages in malignancies that have disseminated into tissues surrounding the primary tumor.
Drug Treatment Plan

Fig. 1: Gastric cancer cells treatment plan. The gastric cancer cells regimen was based on a planned Phase II Study of Low Dose Fractionated Whole abdomen Radiation Therapy (LDFRT) for patients with peritoneal carcinomatosis from gastric or gastroesophageal junction primary adenocarcinomas. The chemotherapy doses were adjusted to produce 50% survival in a cell based assay. The gastric cells were exposed to different radiation doses for three consecutive days (Day 1 to 3). On day 1 the cells were exposed to radiation, 5-fluorouracil (5FU; 3.8 μM) and Docetaxel (DTX; 0.5 mM). On day 2 the cells were exposed to radiation and 5FU (3.8 μM). On day 3 the cells were exposed to radiation, 5FU (3.8 μM) and Cisplatin (CDDP; 1.6 μM). On day 4 the cells were either analyzed for viability, RNA profiling or allowed to grow further to assess survival on day 10.
Cell Viability and Survival

Metastatic gastric carcinoma

A. WST-1 NCI-N87

Gastric adenocarcinoma

B. WST-1 AGS

C. 5822

D. 1739
The mDCF Enhancement Ratio by Radiation is >1 at every radiation doses, indicating that radiation enhanced the killing efficiency of mDCF.
Nitric Oxide Pathway PCR Array
DUOX2 Possible Mediator of HRS

- Dual oxidase is an enzyme involved in:
  - Production of ROS
  - Generation of H₂O₂

- Dual oxidase:
  - Peroxidase homology domain
  - GP91PHOX (NADPH-oxidase) domain

- Expressed in tissues affected by radiation:
  - Thyroid gland
  - Airway epithelial cells
  - Salivary gland
  - Gastrointestinal tract

Table 1:

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<thead>
<tr>
<th>Gy</th>
<th>RRER shDUOX2/SC</th>
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<tr>
<td>0</td>
<td>1</td>
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<tr>
<td>0.15</td>
<td>2.42</td>
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NCI-N87

% Cell Viability vs Radiation Doses (Gy)

NCI-N87

% Cell Viability vs XR + Drugs

DUOX2

GAPDH
LDFRT induced ROS Formation

Cells were labeled with the oxidation sensitive probe H2DCFDA that can be oxidized to its fluorescent product DCF by hydroperoxides and other prooxidants.

Combined regimen produced the highest levels, up to 3.5 times more, as compared to untreated cells

NAD(P)H oxidase inhibitor DPI (diphenylene iodonium), significantly decreases fluorescent levels and the cell killing efficiency of LDFRT combined with mDCF.
Only two genes, Lactoperoxidase (LPO) and Amiloride Binding Protein 1 (ABP1) were upregulated to higher levels by the combined treatments than by each individual modality. LPO uses hydrogen peroxide as a substrate and can contribute to oxidative stress, ABP1 also known as diamine oxidase, is a biomarker for 5-FU GI toxicity.
Genes usually associated with the classical DNA damage response such as ATM, TP53, RB1 and BRAC1 were either down regulated or not affected by the combined regimen in both gastric cell lines and in normal epithelial intestinal cells FHsInt74.

CDKN1A/p21, APAF1 and ANGPT2, were upregulated to higher levels by the combined treatment than by each individual modality. Some of these genes are likely to contribute to chemopotentiation by LDFRT by promoting apoptosis. p21 can increase ROS levels and induce cell death in certain cancer cells, the production of Hydrogen peroxide by DUOX2 could promote an interaction between caspase-9 and apoptotic protease-activating factor 1 (Apaf-1) and upregulation of ANGPT2 can activate caspase-3.
DUOX2 could be used as a biomarker for chemopotentiation by LDFRT

Activation of DUOX2 could lead to protein oxidation in the blood

OxiSelect™ Protein Carbonyl ELISA Kit
(Cell BioLAb, Inc)

Derivatized with dinitrophenylhydrazine (DNPH).

Down regulation of DUOX2 (sh-DUOX2) prevents serum protein oxidation in the media of cells exposed to 0.15 Gy
DUOX2 could be used as a biomarker for chemopotentiation by LDFRT

DUOX2 associated with inflammation

Strong staining of DUOX2 in the surface epithelial cells of 86% (6/7) cases of gastritis

Qi R et al, Gastroenterology Research and Practice Volume 2016, p1-7
DUOX2 could be used to stratify patients for chemopotentiation by LDFRT

~ 50% of gastric tumors are DUOX2 negative
37 gastric cancer samples

<table>
<thead>
<tr>
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<th>N0 Tumors</th>
<th>%</th>
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<tr>
<td>Negative</td>
<td>19</td>
<td>51</td>
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<tr>
<td>Weak</td>
<td>2</td>
<td>5</td>
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<tr>
<td>Positive</td>
<td>16</td>
<td>43</td>
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The possibility to irradiate the whole abdomen with LDFRT without limiting standard of care could provide a new treatment paradigm for disseminated GI tumors and could potentially improve survival.

- LDFRT can potentiate mDCF in gastric cancer cell lines
- The mechanism(s) underlying LDFRT in these cells could include up regulation of DUOX2 and down regulation of conventional DNA repair mechanisms
- DUOX2 leads to ROS formation and induces hyperradiosensitivity

- The possibility to irradiate the whole abdomen with LDFRT without limiting standard of care could provide a new treatment paradigm for disseminated GI tumors and could potentially improve survival.
Questions ????